

disclaimer.

Attached hereto is Appendix A which is the marked-up version of claims 14, 24, 25 and 34.

REMARKS

Claims 14, 24, 25, and 34 have been amended to define the invention with more clarity and particularity. Claims 23 and 33 have cancelled without prejudice or disclaimer. No new matter has been added to the specification as a result of the amendments. Claims 14-22, 24-32 and 34 remain before the Examiner. Applicants respectfully request reexamination and reconsideration of those claims in their presently amended form.

Objections

At pages 2 and 3 of the Action, the Examiner has set forth objections relating to priority claims, Information Disclosure Statements and trademarks. Applicants respectfully request that correction of these objections be held in abeyance until claims are allowed.

Rejections under the First Paragraph of 35 U.S.C. § 112

The Examiner has rejected claims 22 and 32 under the first paragraph of 35 U.S.C. § 112 for an alleged lack of enablement. In particular, the Examiner asserts that hybridoma ATCC 75408 is required to practice the claimed invention and that an affidavit or declaration is needed to assure removal of access restrictions following issuance of a patent. Enclosed herewith is the deposit receipt indicating that the deposit shall be available once a patent issues. In light of that receipt, this rejection is now moot.

Rejections under the Second Paragraph of 35 U.S.C. § 112

The Examiner has rejected claims 14-22 and 25-32 under the second paragraph of 35 U.S.C. § 112 as omitting needed steps and claims 14-34 as allegedly being indefinite for failing to precisely define "framework". Applicants respectfully argue against this rejection for the following reasons.

Applicants point out that the method of claims 14 and 25 need only comprise the recited steps. Other steps can be included. To expedite prosecution, however, claims 14 and 25 have been amended to include the additional steps of original claims 23 and 33, respectively. In light of these amendments, this rejection is now moot.

The phrase "framework region" as used in the context of immunoglobulin molecules (e.g., antibodies) is well known in the art. Enclosed herewith as Exhibit A are excerpts from a textbook entitled, "Fundamental Immunology", edited by William E. Paul, M.D., copyrighted in 1984. It can be seen from those excerpts that the phrase "framework region" is a well defined term known to those of skill in the art. In view of this art recognized definition, Applicants respectfully submit the use of the phrase "framework region" in the claims of the instant application is not indefinite and that one of ordinary skill in the art would readily appreciate the meaning of that phrase.

Rejections under 35 U.S.C. § 103

The Examiner has rejected claims 14, 17-19, 25 and 28-30 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Caiman and Rajewsky (hereinafter "Cumano") in light of Higuchi. The Examiner asserts that Cumano teaches a method of producing an antibody combining site using oligonucleotides that have 3' and 5' termini, framework regions and an $[\text{NNK}]_n$ sequence as recited by the claims. Applicants argue against this rejection for reasons

set forth below.

The rejected claims provide a method for producing an antibody combining site using induced mutagenesis of a CDR region using an oligonucleotide primer having a 5' portion that hybridizes to a framework region of an immunoglobulin light chain, a mutagenic portion defined by the formula $[\text{NNK}]_n$, and a 3' portion that hybridizes to a framework region of the same immunoglobulin light chain gene. The Cumano cited art does not teach, suggest or even mention the use of oligonucleotide primers for mutagenizing CDR portions of immunoglobulin light chain gene. Contrary to the assertions of the Examiner, the sequences set forth in Figures 1 and 2 of Cumano are not oligonucleotide primers. Rather they represent nucleotide sequences of portions of antibodies isolated from particular hybridomas. To make a sequence of Figures 1 and 2 of Cumano read on the presently claimed invention, it is necessary to remove triplet codons from the sequences of both Figure 1 and Figure 2. By way of example, to make the V γ 1 sequence of Figure 1 read on claim 1, the Examiner found it necessary to remove the codon "TCA" at the beginning of the CDR 1 region as well as condons "ACA et seq." from that same CDR 1 region. Similarly in Figure 2, the Examiner has found it necessary to remove from the V186.2 sequence, triplet codons beginning with TAC et seq.

The cited Cumano art does not teach mutagenesis whatsoever let alone the use of a particular triplet codon for accomplishing such mutagenesis. Cumano is nothing more than disclosure of nucleotide sequences of portions of antibodies obtained from hybridomas and/or immunized animals. Cumano cannot be viewed as teaching any process for mutagenizing an immunoglobulin light chain gene, nor the use of any oligonucleotide primers for accomplishing such mutagenesis.

The deficiencies of Cumano cannot be cured by Higuchi, which

is cited by the Examiner as disclosing only that PCR can be used to introduce alterations into DNA. That is, a combination of Cumano and Higuchi does not lead an artisan to the presently claimed invention.

In view of the above, Applicants respectfully request the rejection of the claims on the basis of Cumano be withdrawn.

SUMMARY

In light of the amendments to the claims and for the reasons set forth above, Applicants respectfully submit that the claims are now in a condition of allowance. An early notification to that effect is hereby earnestly solicited.

Respectfully submitted,

8-27-01

DATE

By: 

Thomas E. Northrup

THE SCRIPPS RESEARCH INSTITUTE
Office of Patent Counsel
10550 North Torrey Pines Road
Mail Drop TPC 8
La Jolla, California 92037
(858) 784-2937

☒ [X] Attorney or agent of
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APPENDIX A

Marked-Up Version of Amended Claims

14. A method for producing an antibody combining site in a polypeptide comprising inducing mutagenesis in a complementarity determining region (CDR) of an immunoglobulin light chain gene which comprises amplifying a CDR portion of the immunoglobulin gene by polymerase chain reaction (PCR) using a PCR primer oligonucleotide, said oligonucleotide having 3' and 5' termini and comprising:

a) a nucleotide sequence at said 3' terminus capable of hybridizing to a first framework region of an immunoglobulin gene;

b) a nucleotide sequence at said 5' terminus capable of hybridizing to a second framework region of an immunoglobulin gene; [and]

c) a nucleotide sequence between said 3' and 5' termini according to the formula:

$[\text{NNK}]_n$,

wherein N is independently any nucleotide, K is G or T, and n is 3 to about 24, said 3' and 5' terminal nucleotide sequences having a length of about 6 to 50 nucleotides, or an oligonucleotide having a sequence complementary thereto[.] ;

d) isolating the amplified CDR to form a library of mutagenized immunoglobulin light chain genes;

e) expressing the isolated library of mutagenized light chain genes in combination with one or more heavy chain genes to form a combinatorial antibody library of expressed heavy and light chain genes; and

f) selecting species of said combinatorial antibody

library for the ability to bind a preselected antigen.

24. The method of claim [23] 14 wherein said one or more immunoglobulin heavy chain genes is a library of heavy chain genes.

25. A method for producing an antibody combining site in a polypeptide comprising inducing mutagenesis in a complementarity determining region (CDR) of an immunoglobulin light chain gene which comprises amplifying a CDR portion of the immunoglobulin gene by polymerase chain reaction (PCR) using a PCR primer oligonucleotide, said oligonucleotide having 3' and 5' termini and comprising:

a) a nucleotide sequence at said 3' terminus capable of hybridizing to a first framework region of an immunoglobulin gene;

b) a nucleotide sequence at said 5' terminus capable of hybridizing to a second framework region of an immunoglobulin gene; and

c) a nucleotide sequence between said 3' and 5' termini according to the formula:

$$[MNN]_n,$$

wherein N is independently any nucleotide, M is A or C, n is 3 to about 24, said 3' and 5' terminal nucleotide sequences having a length of about 6 to 50 nucleotides, or an oligonucleotide having a sequence complementary thereto[.] ;

d) isolating the amplified CDR to form a library of mutagenized immunoglobulin light chain genes;

e) expressing the isolated library of mutagenized light chain genes in combination with one or more heavy chain genes to form a combinatorial antibody library of expressed heavy

and light chain genes; and

f) selecting species of said combinatorial antibody library for the ability to bind a preselected antigen.

34. The method of claim [33] 25 wherein said one or more immunoglobulin heavy chain genes is a library of heavy chain genes.



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INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

The Scripps Research Institute
Attention: Michael T. White, Ph.D.
Office of Technology Transfer, Industrial Liaison Officer
10686 North Torrey Pines Road, TPC 9
La Jolla, CA 92037

Deposited on Behalf of: The Scripps Research Institute

Identification Reference by Depositor:

ATCC Designation

Plasmid, TSR334.0 - pC3AP313
Plasmid, TSR334.0 - p7EII

75408
75409

The deposits were accompanied by: ☐ a scientific description ☐ a proposed taxonomic description indicated above.

The deposits were received February 2, 1993 by this International Depository Authority and have been accepted.

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If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

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Bobbie A. Brandon, Head, ATCC Patent Depository

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FUNDAMENTAL IMMUNOLOGY

Editor

William E. Paul, M.D.

*Laboratory of Immunology
National Institute of Allergy and
Infectious Diseases
National Institutes of Health
Bethesda, Maryland*

Raven Press ■ New York

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A fraction of the cells that proliferate on stimulation with antigen differentiate into antibody-secreting cells. Several morphological types of antibody-secreting cells are recognized, of which the best known are the plasma cells.

B Cell Subsets

Two major B cell populations have been demonstrated in the mouse (Fig. 1). They differ from one another in the membrane antigens they express, the immunogens against which they respond, and the nature of the regulatory processes that control their responses. One class of B cells bears the Lyb 5 antigen; these cells are referred to as Lyb 5⁺ B cells. Lyb 5⁺ B cells appear to be mainly responsible for antibody responses to soluble polysaccharides, such as the capsular polysaccharides of pyogenic bacteria, and to hapten conjugates of such polysaccharides (Table 1). A hapten is a low molecular weight (MW) substance that is not by itself capable of initiating an immune response; however, antibodies can be made against it if it has been conjugated to an immunogenic molecule. T cell help for Lyb 5⁺ B cells can be mediated by soluble nonspecific lymphokines, such as BCGF and a set of differentiation factors (Chapter 21). Much of our current understanding of the functions of Lyb 5⁺ B cells derives from studies of mice with an X-linked immunodeficiency ("xid" mice) that lack this B cell subpopulation.

Lyb 5⁻ B cells respond to soluble protein antigens, many cellular antigens, and certain products with intrinsic B cell mitogenic capacity, such as bacterial lipopolysaccharide (LPS). They are unresponsive to

soluble polysaccharides. The mechanisms through which Lyb 5⁻ B cells receive help from T cells appear to be quite different from those of Lyb 5⁺ B cells. Lyb 5⁻ B cells appear to require a direct physical interaction with a T cell specific for the same antigen for which the B cell is specific. Such T cell-B cell interactions are often referred to as cognate help (Chapter 18). An important property of cognate help is that it displays histocompatibility restriction of cellular interactions (Chapter 15); this phenomenon will be discussed in greater detail below.

IMMUNOGLOBULINS

Structure

Chapter 7

The products of antibody secreting cells are Ig molecules. Igs are a group of proteins that have several structural features in common. They are constructed of one, or several, units, each of which consists of two heavy (H) polypeptide chains and two light (L) polypeptide chains (Fig. 3). Each unit possesses two combining sites for antigen. The H and L chains are made up of a series of domains, each of about 110 amino acids. The L chains, of which there are two major types (κ and λ), consist of two domains. The carboxyterminal domain is essentially identical among L chains of a given type and is referred to as the constant (C) region. The aminoterminal domain of L chains varies from antibody to antibody. This domain represents the L chain's contribution to the binding site of the antibody molecule. Because of its variability, it is referred to as the variable (V) domain. The variability of this domain is actually concentrated in three segments of the region, designated the hypervariable or complementarity determining regions (CDR). The CDRs appear to contain the amino acids that line the antibody's combining site. The three CDRs are interspersed in four regions of much lower degrees of variability, designated framework regions (FR). Immunoglobulin L chain (and H chain) V regions can be classified into groups based on similarities in the structure of their FRs.

The H chains of immunoglobulin molecules are of several types, including μ , δ , γ (of which there are several subclasses), α , and ϵ . An assembled immunoglobulin molecule, which consists of one or more units of two identical H and L chains, derives its name from the H chain that it possesses. Thus, there are IgM, IgD, IgG, IgA, and IgE antibodies. The H chains each consist of a single amino terminal V domain and several (generally three or four) C domains. In many H chains, a hinge region separates the first

Table 1. Mechanisms of T cell help

Type of help	Histo-compatibility restricted	B Cell involved	Types of antigens
Cognate	Yes	Lyb 5 ⁻ (?Lyb 5 ⁺)	Thymus-dependent (TD) (e.g., soluble proteins, cellular antigens)
Factor dependent	No*	Lyb 5 ⁺	Type 1 (e.g., polyclonal B cell activators [LPS])
			Type 2 (e.g., soluble polysaccharides)
			Some TD antigens

* T cell-B cell interaction is not histocompatibility-restricted. However, in "factor dependent" responses, T cell activation by APC is histocompatibility-restricted.

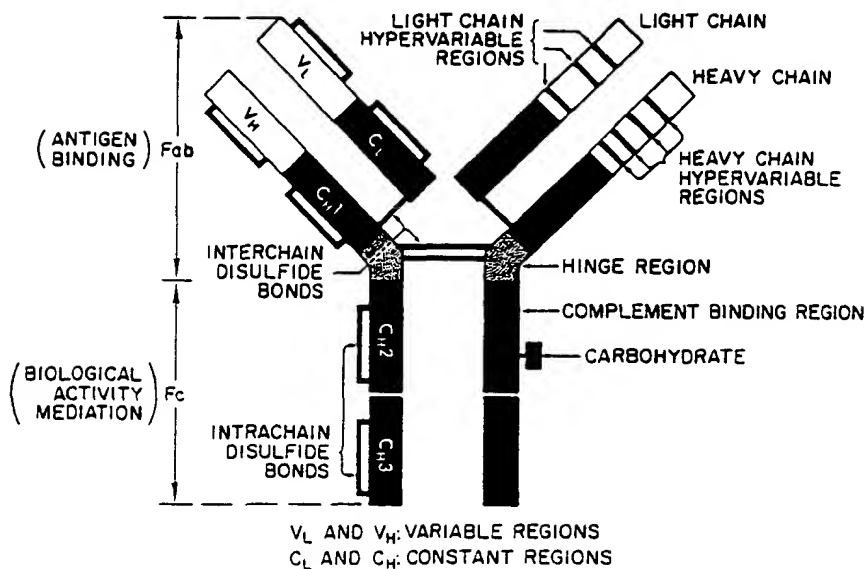


FIG. 3. Structure of an Ig molecule. A schematic representation of an IgG molecule indicating the chain and domain structure of the molecule and the existence of hypervariable regions with variable regions of both H and L chains. (This figure also appears in Chapter 8, where it is explained in greater detail.) [From Wasserman, R. L., and Capra, J. D.: *Immunoglobulins*. In: *The Glycoconjugates*, edited by M. I. Horowitz and W. Pigman pp. 323-348. Academic Press, New York, 1977, with permission.]

and second C domain and conveys flexibility to the molecule, allowing the two combining sites of a single unit to move in relationship to one another. The H chain V region, like that of the L chain, consists of three CDR, which line the combining site of the antibody, and four FR. Thus, the antigen-combining site of an individual antibody molecule is created by contributions from specialized regions of both the H and L chain V regions. The H and L chain V regions, because of their structural uniqueness, can themselves act as antigenic determinants (Fig. 4). Such immunoglobulin V region antigenic determinants are designated idiotopes and the collection of idiotopes on any antibody molecule is its idiotype (Chapter 9). Idiotopes can be constructed of conformations on H or L chain V regions only or, as seems to be true in a majority of cases, of contributions from both chains. Some idiotopes are closely related to the antigen-combining site and the occupation of the site of antigen prevents anti-idiotope antibody from binding to the immunoglobulin. Such idiotopes are designated "site-related" idiotopes. Other idiotopes appear to be less closely related to the combining sites. T cells and antibodies specific for idiotopes may play an important role in the regulation of the immune system (Chapter 22). An influential theory (the network theory of the immune response) has proposed that they may be the critical regulatory elements of the system.

The C region of each H chain class differs from those of the other classes and is responsible for the distinct biologic functions of each class of antibody: (a) IgM antibodies can activate the complement system (Chapter 24); (b) IgA antibodies are secreted into a variety of bodily fluids and provide secretory immunity; (c) IgE antibodies fix to specific receptors on

mast cells and basophils and, when they are cross-linked by antigens, cause these cells to release their biologically active products which result in allergic-type phenomena (Chapter 27); (d) IgD antibodies act almost exclusively as membrane receptors for antigen (Chapter 10); and (e) the IgG antibodies express a variety of functions, including the capacity to be transferred across the placenta.

IgD, IgG, and IgE antibodies generally consist of a single unit of two H and two L chains. IgM antibodies are constructed of five such units, although they consist of a single unit when they act as membrane receptors, and IgA antibodies may consist of

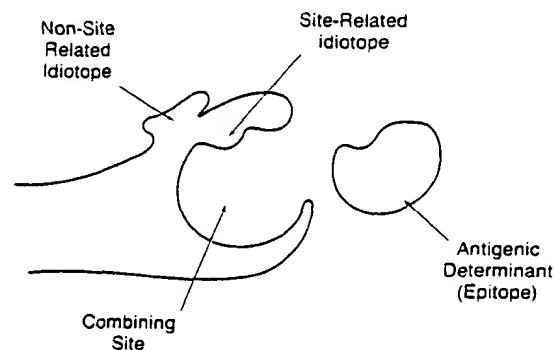


FIG. 4. Idiotopes. Antibody molecules possess combining sites through which they bind antigenic determinants (epitopes). Since distinct antibodies differ from one another in their variable regions, each possesses structures that may themselves be antigenic and elicit the production of antibodies. The antigenic determinants of the variable regions of antibodies are idiotopes. Idiotopes may actually be within the combining site of the antibody (site-related idiotopes) or may be outside the combining site (non-site-related idiotopes).

Chapter 7

Immunoglobulins: Structure and Function

Debra J. Jeske and J. Donald Capra

Department of Microbiology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235

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The major function of immunoglobulin molecules is to bind specifically to foreign or nonself molecules (antigen) and to effect the inactivation and/or removal of that toxin, microorganism, parasite, or other substance from the body. To achieve this, the organism has the capacity to produce a vast array of structurally similar yet individually unique immunoglobulins. The analysis of the structure of immunoglobulins during the last 25 years has been one of the most fruitful undertakings in modern biology, and has led to fundamental advances in the elucidation of protein structure-function relationships as well as to the dissection of immunoglobulin gene organization. This chapter summarizes the current view of immunoglobulin structure. For the most part, human molecules are used as examples since the data from the human is most complete, but data from other species, particularly the mouse, will also be discussed.

Antibodies in their monomeric form are four-chain macromolecules containing two identical heavy chains and two identical light chains per molecule, as shown schematically in Fig. 1 (1). The four chains are covalently linked by disulfide bonds. Each chain is made up of a variable (V) region and a constant (C) region (2). Enzymes such as papain will split the molecule

into three fragments. Two of these, designated Fab, contain the variable regions and retain the antigen binding function of the immunoglobulin. The third, designated Fc, is composed of part of the constant region and retains the effector functions such as complement binding and binding to leukocyte cell receptors (3).

The three-dimensional structure of an intact immunoglobulin is shown in Fig. 2. The molecule is made up of compact globular regions (4). Even before X-ray crystallographers solved this structure, Edelman had proposed that such regions would exist. He noticed that the primary amino acid sequence of immunoglobulin heavy chains could be divided into homology units of 110 amino acids each. He hypothesized that each of these homology units, or domains, had evolved to serve a specific function, a notion which has gained the weight of experimental evidence with the passage of time, as will be amplified later in this chapter when functional aspects of immunoglobulins are discussed (5).

There are two domains in light chains (one variable region and one constant region) and four or five in heavy chains, depending on the class of heavy chain. Each of these segments bears a characteristic tertiary

are equally likely to occur. For example, at position 24 of the $\kappa 1$ subgroup, glutamine is found in about half of the proteins and arginine in the other half (see Fig. 24). There is no evidence of allelism associated with this position since both amino acids are present in all individuals. This and other similar analyses suggest further subdivision of the subgroups.

The subgroups of kappa and lambda chain variable regions are distinct. Thus, no kappa subgroup is found in association with a lambda-type C region and no lambda subgroup is ever associated with a kappa C region. As mentioned above, heavy chain variable regions can also be divided into subgroups that are distinct from kappa and lambda subgroups. Thus, each constant region family (kappa, lambda, heavy) associates with a distinct pool of V region genes (64).

Framework and Hypervariable Regions

The sequence variability of the V region is not random but is organized on a surprisingly precise basis. Immunoglobulin V regions all contain hypervariable regions in which the sequence variation from protein to protein is especially marked. Figure 25 shows several human heavy chains of a single V region subgroup ($V_H III$). Four segments can be termed hypervariable. These include positions 31–37, 51–68, 84–91, and 101–110. Among these, the first, second, and fourth hypervariable regions have been termed complementarity determining because X-ray diffraction and affinity-labeling studies (see below) of immunoglobulins have shown that these segments do, indeed, line the antibody combining site (65). However, although variability at positions 31–37, 51–68, and 101–110 can be related to antigen binding, the function or significance of the heavy chain hypervariable region between positions 84 and 91 is not yet clear (66). It is conceivable that this segment reflects some important V region genetic polymorphism.

The hypervariable regions can be clearly seen by graphing a parameter termed variability which was introduced by Wu and Kabat (67) and defined as follows: variability equals the number of different amino acids at a given position divided by the frequency of the most common amino acid at that position. The denominator is the number of times the most common amino acid occurs divided by the number of proteins examined. Thus, for example, in Wu and Kabat's original study, at position 7 of light chains, 63 proteins had been sequenced and serine occurred 41 times. Four different amino acids, proline, threonine, serine and aspartic acid, had been detected at this position. The frequency of the most common amino acid was thus $41/63 = 0.65$ and the

variability was 4 divided by $0.65 = 6.15$. In this equation, an invariant residue would have a variability of one, whereas the theoretical upper limit for 20 amino acids randomly occurring at any particular position would be $20/1/20$ or 400. The variability profile for the variable region of human heavy chains (based on approximately four times as much data as is shown in 25) is shown in Fig. 26. One observes that the "variable" region is relatively "constant" except in the "hypervariable" areas.

The position of the complementarity determining hypervariable regions in both the heavy and light chain is the same in all vertebrates. This common location for hypervariable regions in immunoglobulins of a variety of animal species suggests that there is a selective advantage in this particular mechanism of generating antibody diversity.

The relatively invariant segments of the V region that are not included in the hypervariable regions (comprising approximately 80–85% of the total V region) are termed the framework regions. As shown by X-ray diffraction analysis, these segments provide a superstructure for positioning the complementarity determining residues in an appropriate position to make contact with antigen. Within a given V region subgroup, variation in the framework segment is very modest (on the order of 5%). There is now no doubt that the framework segments of the V regions are responsible for the generally similar three-dimensional structure of the combining region of all antibodies. The framework segments have very similar dimensions in immunoglobulins from various higher animal species that have been studied to date, again, indicative of the general biological unity reflected in this component of the humoral immune response.

The Antibody Combining Site

Affinity-labeling studies of purified antibodies demonstrate that the hypervariable regions are, indeed, those portions of the molecule containing the residues that make contact with antigen and, therefore, determine antigenic specificity. Both chains participate in antigen binding since the affinity label is generally found in both heavy and light chains. The amino acid sequence of affinity-labeled peptides revealed that the labeled sites were near or within hypervariable regions although generally only a few hypervariable regions were ever implicated in a single study (see Fig. 27).

The three-dimensional structure of an Fab fragment (Fig. 28) gives a precise view of a combining site with hypervariable region sequences clustered spatially at one end of the molecule and fully exposed to solvent. The crystallographic structure of ligand-